Author's response to reviews

Title: Expression of TLR3 and its correlation with apoptosis, proliferation, angiogenesis and prognostic in HCC

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Author's response to reviews: see over
Dear editor,

My manuscript, Expression of TLR3 and its correlation with apoptosis, proliferation, angiogenesis and prognostic in HCC, was revised according to the viewers’ comments, and the itemized response to each reviewer’s comments is attached. Many thanks to your suggestion. I am so sorry to bring you so much trouble because of our careless.

Correspondence about this paper should be directed to Li Chen at the following email address:bl1@ntu.edu.cn. Thanks very much again for your attention to our paper.

Once again, thank you for your help to our paper processing.

For your guidance, itemized response to each reviewer’s comments is appended below.

Dear Reviewer1

1. This manuscript is not well written and the authors need to amend their grammatical mistakes.
   As a Chinese, our English is very poor and a few of corrections and editing had done by an English teacher.

2. How many HCC cases are HBV-related? Any correlation between HBV status with the TLR3 signaling?
   We have added a few content of correlation between HBV infection with TLR3 expression.

3. Survivin and Bcl-2 are known to be direct transcriptional targets of NF-kB. However, Survivin and Bcl-2 was found to be negatively correlated to the expression of NF-KB. Can author explain this discrepancy?
   There are several reasons. First, the number of the cases in this study is small, the result may be biased. Second, TLR3 signaling may induce two downstream pathways, the inflammatory or the apoptotic pathway. The inflammatory pathway is mediated mainly by Rip1 and leads to NF-κB activation. The apoptotic pathway, on the other hand, was shown to be mediated by Rip3 and results in caspase-8 activation. Several studies have shown that in human hepatoma cell lines, unlike white blood cells, TLR3 signaling is skewed towards the apoptotic pathway. A similar observation was also reported in other tumor cells. In this study, we thought that TLR3 signaling maybe towards the apoptotic way rather than activate NF-κB. That is why Survivin and Bcl-2 was found to be negatively correlated to the expression of NF-KB.
Dear Reviewer2  
Major comments:  
1. All the data authors presented are subjective and descriptive, therefore, it is necessary to add some material data, such as computer-aided image assessment for semi-quantitative assay of positive staining area in the slides.  
   Stained sections were evaluated in a blinded manner by two independent investigators without prior knowledge of the clinical information.

2. Most of the coefficients of correlation are less than $\pm 0.5$, indicating that expression of TLR3 has weak correlations with the parameters of cellular apoptosis and proliferation, etc. These may be improved by dual immunohistochemical stainings, such as TLR3 + TUNEL, TLR3+Ki-67 and TLR3+NF-kB, etc. As you suggested, we did dual immunohistochemical stainings, but our method was not yet brought into a ripe condition. The results were similar with the original results. However, these are our preliminary study results and we will detect more tumor markers in our future in-depth research.

3. In HCC cells, it was found that cell surface stimulation of TLR3 with Poly I:C did not affect cell viability, in contrast, cytoplasmic stimulation with transfected Poly I:C significantly induced apoptosis accompanied by the down-regulation of anti-apoptotic protein. The question is that did authors find some differences in correlations of TLR3 with apoptosis between membrane and cytoplasmic staining patterns? We have added a few content of expression pattern of TLR3 in HCC tissues and the correlation of TLR3 expression pattern and apoptosis.

Minor comment:  
In all microscopic figures, authors only provided high magnification (X400) views, in order to understand more global views of distribution of positive stainings, less magnificent view pictures should be showed.  
Figures are changed.